

Sow milk composition changes over lactation, and modulation of maternal diet may enhance intestinal epithelial cell membrane composition to enhance gut barrier function in the suckling neonatal piglet.

Abstract:

Dietary nutrients are essential for gastrointestinal (GI) growth and function, and a significant component of neonatal development requires the nutritional support of GI growth and development. Nutritional provisions of the mother's milk support normal maturation of structure and function of the GI tract in most neonates. The composition of mother's milk affects GI, mucosal immune system, and neurological development. The functional nutrients and other bioactive components of milk support a microenvironment for gut protection and maturation. However, early intestinal maladies can impair normal GI development, leading to intestinal dysfunction and even death. Therefore, our study evaluated sow colostrum and milk composition of the bioactive phospholipids of the milk fat globule membrane, as well as, milk protein composition to gain knowledge about potential ways to modulate maternal diet to enhance gut barrier function of suckling piglets, a dual purpose agri-medical model. Experiment one collected colostrum and mature milk from 11 first parity sows at 12-24h and 8-10d post-partum, respectively. The percent total solids were significantly greater in colostrum samples compared to mature milk samples (23.51 vs. 19.29 ± 1.34 ; $P < 0.05$), however total fat was not significantly different between sow colostrum and mature milk (8.93 vs. 8.33 ± 0.78 ; $P > 0.05$). Total phospholipids and milk associated proteins were also greater in colostrum vs. milk. Interest in suckling neonatal piglet intestinal phospholipid composition derive from maternal diet led to the development of a piglet intestinal cell culture model to determine if we could enrichment of LC-PUFA in the cell membrane for developing an in vitro challenge model. In conclusion, we know milk composition changes over lactation and changes in milk solids, specifically phospholipids, may play an important role in modulation of intestinal health in neonates.

Introduction:

Worldwide, there is a shockingly high prevalence of fatal enteric diseases and dysfunction in neonates, both domestic livestock and human infants. Fifteen percent of childhood mortalities are due to diarrheal diseases and 41% occur in neonates (1). In the swine industry, \$2 billion (USD) is lost annually to intestinal diseases, 16% of which occur in the post-weaning stage of life (2). These intestinal maladies that occur across species such as necrotizing enterocolitis and gastroenteritis have led to several studies on neonatal gut function and development in relation to overall growth and wellbeing. However, more information is needed surrounding the role that specific dietary nutrients play in the establishment and maintenance of a functional gastrointestinal tract and a homeostatic mucosal immune system.

Dietary nutrients play a critical role in neonatal intestinal development. Nutritional factors have been implicated in the maturation of the neonatal gut, however, there are still questions as to what factors in maternal milk play critical roles in the development of a homeostatic intestinal environment. The mammary gland packages milk lipids into a unique phospholipid tri-layer which

contains membrane-bound proteins, glycoproteins, enzymes and cholesterol which are known to confer protective effects on the developing neonatal gut (2, 3). The milk fat globule member (MFGM) is composed of bioactive polar lipids and phospholipid bound LC-PUFA that may enhance intestinal membrane integrity and gut barrier function in the development neonatal intestine (2, 3). Therefore, understanding the lipid composition of sow's milk could allow manipulation of maternal diet to enhance piglet intestinal and immunological health. A robust maturation of the gastrointestinal and immune systems that may last throughout the lifetime of the piglet is important to the swine industry due to the various immune challenges presented throughout the lifetime of a production animal and effects on growth.

Additionally, when the diet of the neonate is enriched with fatty acids and their subsequent metabolites, specifically long-chain polyunsaturated fatty acids (LC-PUFA), there is potential for beneficial modulation of biochemical pathways, cell signaling, and immune function. Concerning LC-PUFA, there is a lot of speculation surrounding the balance between n-3 and n-6 LC-PUFA and how various levels directly influence immune, retinal, gut, and brain development. Both n-3 (specifically eicosapentaenoic, EPA) and n-6, (specifically arachidonic, ARA) fatty acids are found throughout the phospholipid membrane of both brain and retinal tissues and play an important role in the inflammatory response by production of eicosanoids. Levels of LC-PUFA in maternal milk depend directly on the mother's diet, demonstrated by previous studies enriching the sow diet with various levels of n-3 LC-PUFA. Maternal supplementation of the sow in these studies translated to decreased villi height, crypt cell depth, and mast cell degranulation as well as increased glucose uptake in piglets (4,5).

In addition to lipids, various proteins in milk such as lactoferrin, albumin, casein, b-lactoglobulin, a-lactalbumin, and immunoglobulins have several possible implications on the healthy growth and development of the neonate. These proteins are proven to have several beneficial actions in the developing neonate such as disrupting microbe adherence to intestinal cells, having bactericidal action, supporting positive microbiome development, and further systemic immune support throughout various pathways of the body.

The objective of these experiments was to investigate sow MFGM lipids and protein composition from colostrum and mature milk, and to determine how dietary phospholipids, fatty acids, and proteins present in the MFGMs secreted by the mammary epithelial cells play an important role in modulation of intestinal health in neonatal piglets throughout the course of lactation. Secondly, to determine if we could enrich piglet intestinal epithelial cells with LC-PUFA to develop and in vitro screening model of microbial disrupting on intestinal barrier function.

Methods

Experiment 1 - sow milk lipid composition: Colostrum and mature milk were collected from 11 first parity sows at 12-24 h and 8-10 d post-partum, respectively. Samples were analyzed for total solids and fat using the SMART Trac II Moisture and Fat Analyzer, and total lipids were extracted by the Folch method followed by the Bitman solid-phase extraction to remove triglyceride (TG) and other neutral lipids (Gallier et al. 2010; Astaire et al., 2003). Phospholipid composition of the samples were analyzed using thin layer chromatography (TLC) (chloroform: methanol: water, 65:25:4, v/v/v) was used to qualitatively identify phospholipids present in the colostrum and milk and check for the presence of trace neutral lipids. 20 uL samples were placed on the plates with a

Hamilton gas-tight syringe (Fisher Scientific). Iodine vapor was applied overnight to reveal spots indicative of various phospholipids. Milk proteins were identified by quantifying total protein in samples by BCA protein assay kit. Following quantification of total protein, 200 mg of total protein/sample were separated by running samples on a 4-20% gradient SDS-PAGE gels (Mini-Protean TGX Stain-free Precast Gels) with a molecular weight marker in order to identify immunologically relevant proteins in the samples. Images were obtained using stain-free settings on a ChemiDoc imaging system.

Experiment 2 - Cell Culture

In experiment two, IPEC-J2, neonatal, pig intestinal cells were cultured in DMEM/F12 media supplemented with 5% FBS, ITS, EGF and antibiotics. Cells were plated at a cell density of 2×10^5 cells/well and grown to 100% confluence. At confluence cell media was supplemented with 30 μ M arachidonic acid and 30 μ M eicosapentanoic acid conjugated to bovine serum albumin for 96 h to enrich the phospholipid membranes of cells with different polyunsaturated fatty acids. Cells were collected at the end of 96 h. Total lipids were extracted from cells and quantified by GC/MS.

Results

The percent total solids were significantly greater in colostrum samples compared to mature milk samples (Fig. 1; 23.51 vs. 19.29 ± 1.34 ; $P < 0.05$), however total fat was not significantly different between sow colostrum and mature milk (Fig. 1; 8.93 vs. 8.33 ± 0.78 ; $P > 0.05$). Total phospholipids and milk associated proteins were also greater in colostrum vs. milk (Fig. 2) and protein concentrations between colostrum and milk samples varied based on protein of interest (Fig. 3).

In addition, interest in suckling neonatal piglet intestinal phospholipid composition derived from maternal diet led to the development of a piglet intestinal cell culture model to determine if we could enrichment of LC-PUFA in the cell membrane for developing an in vitro challenge model. Neonatal piglet intestinal epithelial cells membrane phospholipids enriched from 96 h in with 30 μ M arachidonic acid (ARA) or 30 μ M eicosapentanoic acid (EPA) were increased compared to the control cell by 5-fold and 2-fold, respectively (Fig. 4). Showing nutrient availability can modulate intestinal phospholipid composition.

Conclusions

Milk composition changes over lactation and changes in milk solids, specifically phospholipids and specific proteins may play an important role in modulation of intestinal health in neonates. Our results demonstrate that important developmental components of the neonate's diet, protein and fat, drop significantly in sow's milk from colostrum to mature milk. Previous studies show that the early neonatal period is a critical time for intestinal, immune, and cognitive maturation which can be facilitated by the supplementation of MFGM and its associated bioactive components (6,7). Rapid compositional changes in maternal milk indicate the importance of ensuring the neonate is receiving adequate amounts of colostrum at birth, but also suggest supplementation of these components throughout the course of lactation may result in improved piglet growth, immune function, and gut health. If fatty acid composition of maternal milk can be modulated,

there is potential to see increased immune competency throughout the life of the piglet in response to environmental challenges such as weaning and bacterial disease. This could be an innovative approach to understand the overlying physiology behind bioactive nutrients.

Discussion:

Neonatal nutrition not only provides essential sustenance for growth and development of the infant, but also greatly impacts intestinal, immunologic, and neurological development and health (8). Neonatal gastrointestinal epithelial structure and function abruptly change from birth to weaning so that infants can adapt quickly from consuming milk to solid foods. Nutritional factors have been implicated in the maturation of the neonatal gut; however, there are still questions as to what factors in maternal milk play critical roles in gut development. The prevalence of formula feeding in the U.S. is approximately 90% by 3 months of age in infants even though maximal benefits of breastfeeding are seen out to 6 months to 1 year of age (9). An increase in gastrointestinal disturbances and chronic allergic and autoimmune diseases is correlated with increases in formula feeding. Examining the compositional changes of important bioactive components of sow's milk aids in elucidating improvements in human infant formula as well neonatal piglet nutrition for the swine industry.

As previously stated, dietary fatty acids and colostrum quality greatly impact neonatal piglet gut, immune, and cognitive health and development. Inclusion of LC-PUFA in maternal sow diets creates not only opportunities for improved piglet growth, but also human infant formula development. Further research is needed on the various immune properties of proteins present in colostrum and mature milk, and how the changing composition of these immune components throughout lactation effect neonatal epithelial cells. Understanding how membrane chemistry is associated with inflammation and how the intestine is directly linked to development and health is a unique approach that will allow further development connecting the overlying physiology with bioactive nutrients present in maternal milk.

FIGURE 1

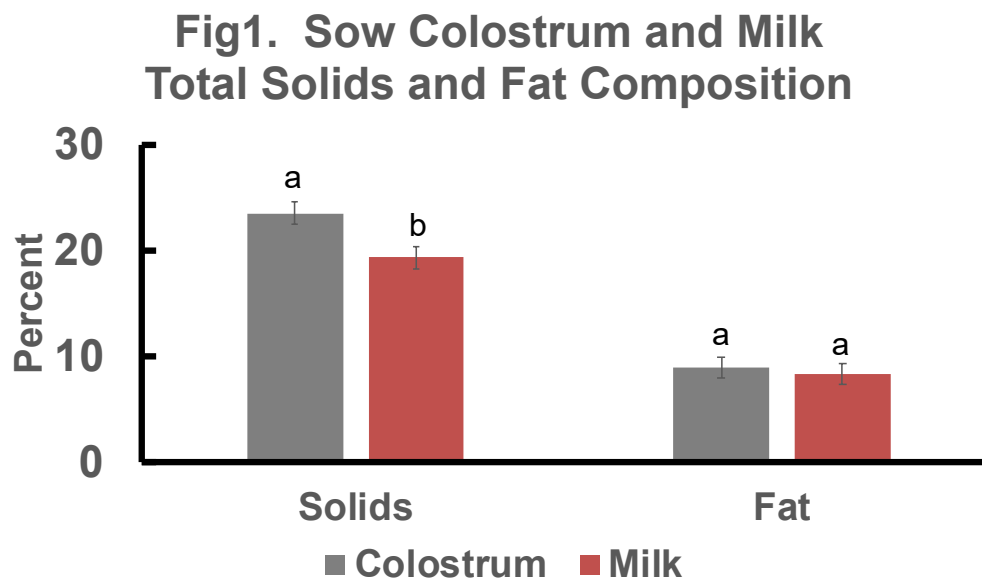


Figure 1. Sow colostrum and milk total solids and fat composition. Different letters denote significant differences at $P < 0.05$ within solids and fat group.

FIGURE 2

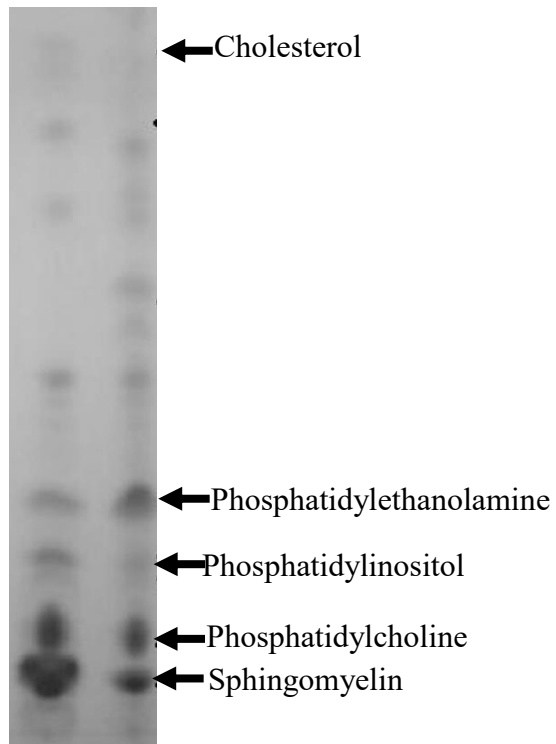
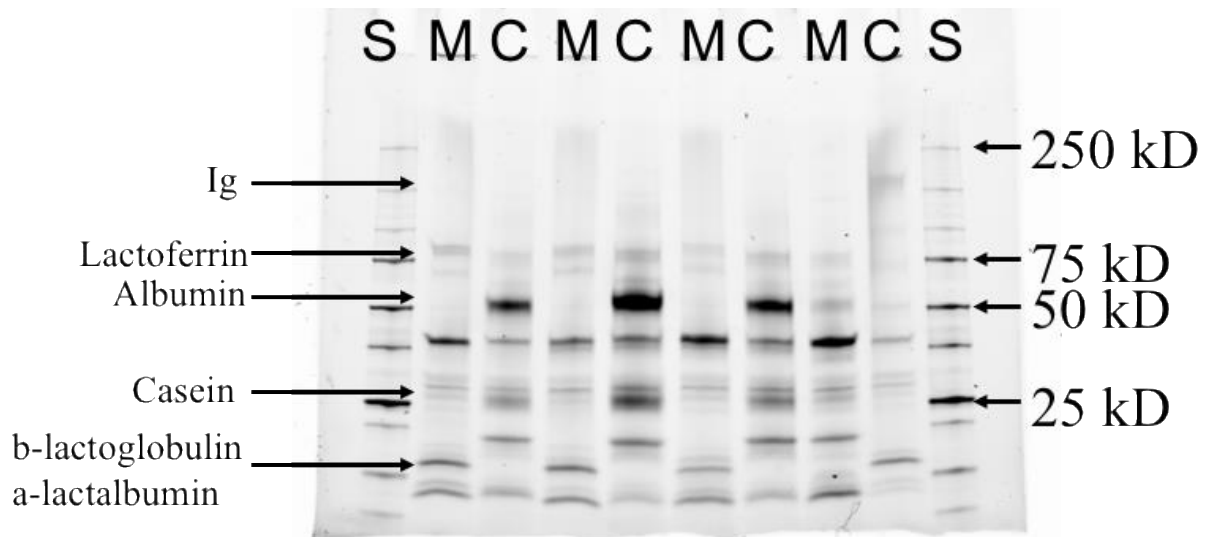


Fig2. Thin Layer Chromatography of Colostrum and Mature Milk Phospholipids

FIGURE 3



S = Standard; M = Mature Milk; C = Colostrum

Figure3: SDS-PAGE Analysis of sow colostrum and milk protein composition.

FIGURE 4

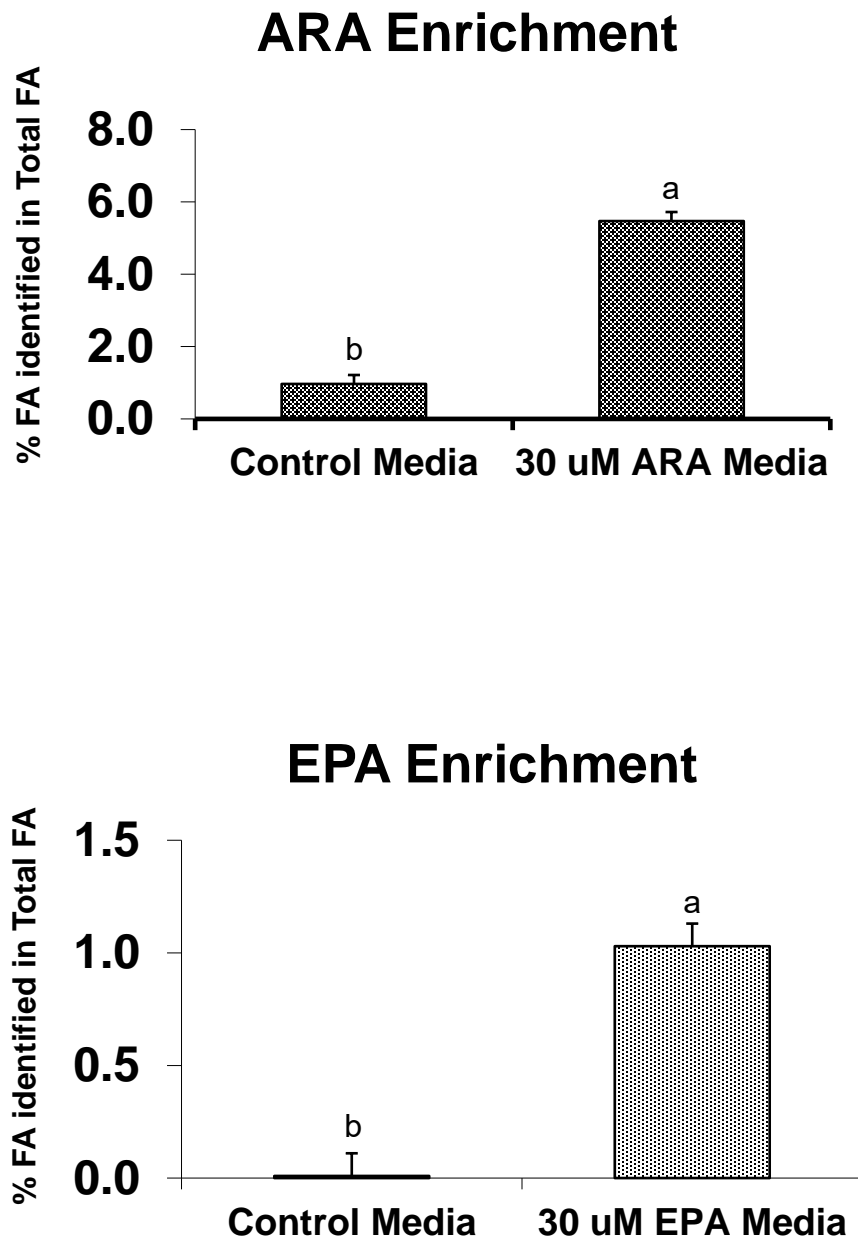


Fig4. Fatty Acid Enrichment of Phospholipid Membrane of IPEC-J2 Cells. Different letters denote significant differences at $P < 0.05$.

Bibliography:

- 1 Hernell et al. (2016) Clinical Benefits of Milk Fat Globule Membranes for Infants and Children. *J Pediatr*; 173S:S60-5.
- 2 Bourlieu, C., K. Bouzerzour, S. Ferret-Bernard, C. L. Bourgot, S. Chever, O. Ménard, A. Deglaire, I. Cuinet, P. L. Ruyet, C. Bonhomme, D. Dupont and I. L. Huërou-Luron (2015). "Infant formula interface and fat source impact on neonatal digestion and gut microbiota." *European Journal of Lipid Science and Technology* **117**(10): 1500-1512.
- 3 Bourlieu, C. and M.-C. Michalski (2015). "Structure–function relationship of the milk fat globule." *Current Opinion in Clinical Nutrition & Metabolic Care* 18(2): 118-127.
- 4 Gallier et al. (2010) Composition and fatty acid distribution of bovine milk phospholipids from processed milk products. *J Agric Food Chem.* 58(19): 10503-10511.
- 5 Castro-Gómez, P., Garcia-Serrano, A., Visioli, F. & Fontecha, J. Relevance of dietary glycerophospholipids and sphingolipids to human health. *Prostaglandins Leukot. Essent. Fat. Acids PLEFA* 101, 41–51 (2015).
- 6 Sprong, R. C., Hulstein, M. F. E., Lambers, T. T. & van der Meer, R. Sweet buttermilk intake reduces colonisation and translocation of *Listeria monocytogenes* in rats by inhibiting mucosal pathogen adherence. *Br. J. Nutr.* 108, 2026–2033 (2012).
- 7 Berding et al. (2016) Prebiotics and Bioactive Milk Fractions Affect Gut Development, Microbiota, and Neurotransmitter Expression in Piglets, *Journal of Pediatric Gastroenterology and Nutrition* 63: 688-697.
- 8 Jacobi SJ, Odle J (2012) Nutritional Factors Influencing Intestinal Health of the Neonate, *American Society for Nutrition. Adv. Nutr.* 3:687-696.
- 9 Astaire JC, Ward R, German JB and Jimenez-Flores R (2003) Concentration of polar MFGM lipids from buttermilk by microfiltration and supercritical fluid extraction, *Journal of Dairy Science* 86: 2297-2307.